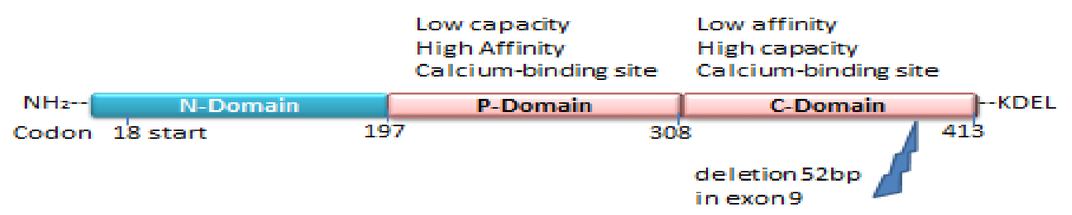
Calreticulin mutation in a case of myopathy

Fabiana Rossi[1], Luca Lombardi[1], Sara Casertano[1], Mario Fratta[1], Raffaele Di Francia[2], Domenico De Lucia[1], Mariarosa AB Melone[1], Simone Sampaolo[1], Giuseppe Di Iorio[1]

1- Second Division of Neurology, University of Campania "Luigi Vanvitelli" – Naples
2- Department of Hematology*, National Cancer Institute, Fondazione "G. Pascale" - Naples*

Introduction

Calreticulin (*CALR*) is a 46 KDa multifunctional Chaperone protein that acts as a major Ca^{2+}-binding (storage) protein in the lumen of the endoplasmic reticulum (ER) regulating calcium homeostasis.
CALR contains two Ca^{2+}-binding sites in the P-domain (high-affinity, low-capacity) and C-domain (low-affinity, high-capacity) and it adjusts storing of more than 50% of Ca^{2+} in ER lumen. Therefore CALR-deficient cells have a lower capacity for Ca^{2+} storage in the ER lumen supporting that CARL should play a critical role in musculoskeletal disorders.
CALR gene somatic mutations have also been used in classification and determination of diagnostic criteria for myeloproliferative neoplasms, including essential thrombocythemia, primary myelofibrosis and refractory anemia



The protein structure could be sub-divided into a globular **domain N-terminal**, a proline-rich **P-domain** forming an elongated arm-like structure and an acidic **domain C-terminal**. The P-domain binds one molecule of calcium with high affinity, whereas the acidic C-domain binds multiple calcium ions with low affinity. The well known somatic mutation in exon 9 are

- **Type 1** a **52-bp deletion** (L367fs*46), which results in the loss of acidic domain and the Lys-Asp-Glu-Leu (KDEL) signal generating a truncated protein with low capacity of Ca^{2+}.bindind
- **Type 2** a **5bp insertion** (K385fs*47), which representes an inverse duplication of the five nucleotides preceding the insertion.

Case report

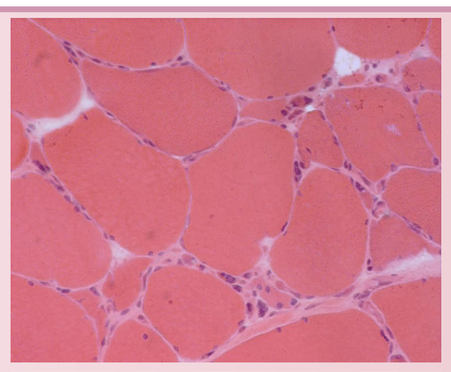
A 20 years-old man, with a diagnosis of Hereditary Spherocytosis (HS), was admitted to our Department complaining exercise intolerance, fatigue, myalgia, cramps and progressive weakness of the lower extremities over 5 years.
Family history was notable for HS on his mother's side (mother, grandfather, uncle, aunt and a cousin) and malignant hyperthermia in a mother's cousin.

Results

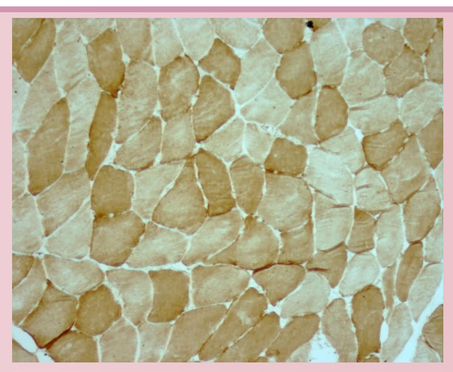
Biochemical investigations revealed: creatine kinase (CK) 50 U/L; 25-hydroxy vitamin D3 (25-OH-D) 21,9 ng/mL; increased total, direct and indirect bilirubin.
Neurological examination, electromyography (EMG) and nerve conduction studies were normal.

Muscle biopsy

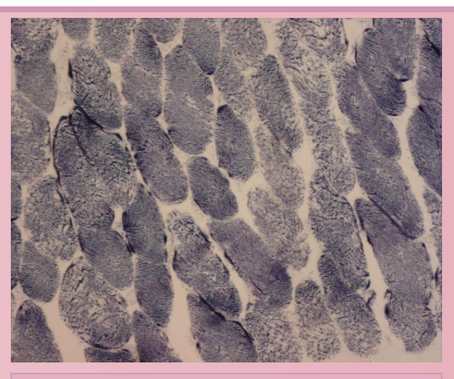
Histology and histochemistry of quadriceps muscle biopsy revealed a myopathic pattern characterized by a moderate variability of fiber diameter with hypertrophic type 2 fibers and rare moth-eaten fibers.
Subsarcolemmal aggregates were observed when stained for SDH, COX and Gomori trichrome.
Immunohystochemical studies revealed faint Spectrin staining. Calpain 12A2 staining was positive in some nuclei.



HEMATOXYLIN EOSIN (20X)



COX (10X)

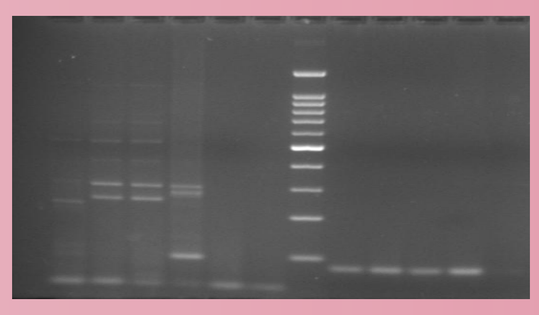


SDH (20X)

ATP pH 9.4 (10X)

Genetic assay

At a two years follow-up, neurological examination and EMG were still negative despite the worsening of symptoms. DNA and RNA samples from Peripheral Blood mononuclear cells (PBMCs) were obtained from the propositus and his relatives. DNA and RNA were also isolated from muscle biopsy.
A panel of candidate genes was performed.
CALR 52-bp deletion Type 1 on exon 9 was identified on muscle-tissue DNA and on DNA from granulocyte population of PBMCs.
Ankirin K1 (Glu713Lys) and Solute carrier organion 1B1 (SLCO1B1) polymorphisms were also found, both at heterozygous state.
Spectrin α and β were genotyped, resulting at a wild type state.



Lane 1: CNT+ DNA con del52
Lane 2: Proband Muscle with 52-bp deletion
Lane 3: Proband WBC with 52-bp deletion
Lane 4: CNT+ DNA with 5-bp insertion
Lane 5+6 CNT- H2O
Lane 6: ladder marker 100bp
Lane 7-10 same DNA of 1-4 amplification. Albumin of control DNA

Discussion and conclusions

Because of the limited knowledge about the association between CALR mutation and myopathy, our case opens a new issue on the 52-bp deletion *CALR.* Moreover, questions regarding the exact contribution of *CALR* deletion in myopathy diseases are still unresolved. A therapy based on supplementation of Carnitine oral solution 2 g/day, vitamin D 150 mcg/day, L-arginine oral solution 1,66 g/day, calcium tablets 500 mg/day was performed with a dramatically reduction of symptoms like fatigue, myalgia and cramps at a 6 month follow-up.
To our knowledge, this is the first case of a patient with a myopathic pattern characterized by a 52-bp deletion in CALR gene.

References

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